First report of non-marine ostracods (Crustacea, Ostracoda) from the Dahlak Archipelago (Eritrea), with the description of two new species

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ABSTRACT

The information on Recent non-marine ostracod faunas of the north-eastern part of the Afrotropical region is still limited. Here we report the first record of ostracods from the Dahlak Archipelago, a group of small islands located in the southern part of the Red Sea off the Eritrean coast. Specimens were obtained by hatching in the laboratory of diapausing eggs occurring in dried mud collected in temporary freshwater ponds, following the so-called "Sars' method". A total of five species were found, including two species new to science, of which four belong to the family Cyprididae (*Cypris galefensis*, *Plesiocypridopsis newtoni*, *Heterocypris* sp., and *Chrissia martensi* n.sp.) and one to the family Ilyocyprididae (*Ilyocypris dahlakensis* n.sp.). A detailed description of the morphology of valves and soft parts is given, and COX1 sequences were obtained for four species. *Cypris galefensis* was so far only known for its type locality in Somalia with a population containing both males and females, whereas our samples consisted exclusively of females. *Plesiocypridopsis newtoni,* a species characterised by a wide geographic distribution and previously known to occur also in the Afrotropical region, shows marked variability in the prehensile palps of males, as evidenced by comparing the specimens from this study with descriptions available in the literature. The genus *Heterocypris* (with 15 species) and the genera of the two new species described here, *Chrissia* (with 17 species) and *Ilyocypris* (with four species), have been previously reported from this biogeographic region as well. The analysed specimens of *Chrissia martensi* n.sp. were all females, none of which had sperm inside the carapace, indicating the possibility of parthenogenetic reproduction in this species. A peculiar sexual dimorphism in the valve morphology characterises *Ilyocypris dahlakensis* n.sp., with females having a straight posterior margin, forming a right angle at the ventral anterior edge. Rehydration of dry sediments collected from arid areas where wet periods are short and often unpredictable has proven to be a successful method for describing aquatic invertebrate biodiversity.

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Key words: recent ostracods; morphology; taxonomy; Sars' method; Dahlak Kebir; temporary freshwater ponds; biodiversity; *Chrissia martensi* n.sp.; *Ilyocypris dahlakensis* n.sp.; COX1 sequences.

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INTRODUCTION

Ostracods (class Ostracoda Latreille, 1802) are bivalve crustaceans characterised by an impressive evolutionary radiation, with species capable of colonising marine ecosystems, inland waters (both surface and subterranean) and semi-terrestrial habitats (Horne *et al.*, 2002; Smith *et al.*, 2015; Mesquita-Joanes *et al.*, 2023). They play an important role as guiding fossils, with an uninterrupted record since the Ordovician for marine forms and the Carboniferous for freshwater ones (Maddocks, 1982; Siveter *et al.*, 2001).

Studies on diversity and distribution of extant non-marine ostracods, all belonging to the order Podocopida (Karanovic, 2012), have covered most biogeographical and bioclimatic regions of the world, and data sets have been constantly growing since the 1980s (Huang *et al.*, 2022). The earliest information on ostracods in freshwater habitats of the Afrotropical region - that includes, following the subdivision adopted by Meisch *et al.* (2024), Sub-Saharan Africa, the southern Arabian Peninsula, the island of Madagascar, and the islands of the western Indian Ocean - primarily stems from scientific expeditions conducted since the late 19th century (see references in Martens, 1982). In more recent times, knowledge about non-marine ostracods from various Afrotropical areas has expanded enormously thanks mainly to the monumental contributions of Koen Martens and his collaborators. Currently, the Afrotropical region is second only to the Palaearctic region in terms of the number of described species of living non-marine ostracods, accounting for about 20% of their global diversity, with an endemism rate of over 80% and a clear predominance of the family Cyprididae (Martens, 1984, 1998; Martens *et al.*, 2008; Meisch *et al.*, 2024).

However, it must be emphasised that there is still considerable variability in the information available for different areas of this biogeographic region. For example, studies carried out in the last decades have described the Recent ostracod faunas of Madagascar (Danielopol and Betsch, 1980; Colin, 2009; Kayo *et al.*, 2012) and East Africa (Rumes *et al.*, 2016), and in particular those of the Rift Valley lakes, which host a considerable portion of the known Afrotropical non-marine ostracod diversity (Martens, 1985; Cohen, 1986; Kibret and Harrison,1989; Martens and Tudorancea, 1991; Schön and Martens, 2012; Jacobs and Martens, 2022). Austral Africa has also been the focus of numerous investigations (Curtis, 1991; Martens *et al.*, 1996; Martens, 1997; De Moor and Day, 2013; Bird *et al.*, 2019; Szwarc *et al.*, 2021, 2023; Szwarc and Namiotko, 2022; Ferreira *et al.*, 2023; Namiotko *et al.*, 2023). For other Afrotropical areas, knowledge about the distribution of living non-marine ostracods comes from occasional studies (Ghetti, 1970; Higuti and Martens, 2016) or is completely absent.

As for the north-eastern part of the Afrotropical region, where the landscape is dominated by arid steppes and deserts (BWh, BSh and BWk climate zones, Beck *et al.*, 2018) and the corresponding freshwater ecoregions have xeric freshwaters and endoreic basins as major habitat types (FEOW, Abell *et al.*, 2008), the available information is still limited. Extant non-marine ostracods have been reported as simple occurrence or identified at different taxonomic ranks from scattered localities of the Horn of Africa (Masi, 1925; Martens, 1982, 1997), Socotra (Mohammed *et al.*, 2004) and the south-central part of the Arabian Peninsula (Victor and Al-Mahrouqi, 1996; Victor and Al-Farsi, 2001; Victor and Victor, 2002; Kardousha, 2016; Saji *et al.*, 2018; Soesbergen, 2018; Al Shidi and Victor, 2022).

In this paper, we report the first record of Recent ostracods from the Dahlak Archipelago, Eritrea, obtained by hatching in the laboratory of diapausing eggs occurring in dried mud collected in intermittent freshwater ponds.

Study area

The Dahlak Archipelago is a group of about 120 islands plus several sand banks and rocky islets situated in the southern portion of the Red Sea (15°50’N, 40°12’E) along the coast of northeastern Africa, which is separated from coastal Eritrea by the Massawa Channel. The islands vary in size from a few hundred square meters to the 644 km^2 of Dahlak Kebir (15°72’N, 40°09’E), the major island of the Dahlak Archipelago, and normally rise from a few meters up to 36 m asl (Isratu island: 16°33’N, 39°88’E).

The carbonate Dahlak islands are flat relicts of large Pleistocene coral reef platforms that formed over salt domes of Miocene origin (Angelucci *et al.*, 1985). Seawaters around the islands are comparatively shallow, not exceeding 150 m at their deepest point. Tides are semi-diurnal and range between 0.5 and 1.2 m (Edwards, 1987).

The climate is hot (annual average temperature in Massawa, approximately 55 km from the sampling sites: 29.0°C) and arid, with showers from November to March (average rainfall 170 mm y^{-1} in Massawa; Nastasi, 1994). The areas above the intertidal zone subject to occasional inundation and saltwater spray are characterized by low bushes of halophytic succulent plants mainly belonging to the genera *Suaeda*, *Zygophyllum* and *Limonium*. The island’s interior is dominated by trees of the genera *Acacia* (in deeper soils) and *Commiphora* (in rocky habitats); *Sterculia Africana* and *Hyphaene thebaica* grow in wetter soils. The undergrowth is formed by bushes of the genera *Salvadora*, *Capparis*, and *Cadaba.* Sparse succulent plants are found of the genera *Euphorbia*, *Cyssus* and *Desmidorchis*. The ground layer is made up of grasses, Poaceae and Cyperaceae, mainly (Terracciano, 1893; Nastasi, 1994; Coulthard, 2001; De Marchi *et al.*, 2009). Mangroves, with *Avicennia marina* as the dominant species, are present in some coastal areas in the north of Dahlak Kebir.

Freshwater is scarce and largely dependent on rainfall. However, on some islands such as Dahlak Kebir, there are several depressions of various shape and size that that fill up with water during limited periods of precipitation (Angelucci *et al.*, 1985).

METHODS

Sediment collection and culturing

On 23 April 2019, three ponds (coded D1, D2 and D3) located on Dahlak Kebir Island, a few hundred meters from the sea shoreline, were visited. All were completely dry on that date. Samples of approximately 500 g of sediment were collected with a shovel from the deepest part of each pond and stored separately in plastic bags. As no crustaceans were obtained after hydration of the sediment from D1, only ponds D2 (lat. 15.67046 N, long. 39.97075 E) and D3 (lat. 15.66986 N, long. 39.97079 E) will be considered henceforth (Fig. 1).

Sediment samples were brought to the laboratory and put in culture following the so-called “Sars’ method”, which is based on the hatching of freshwater crustaceans from their resting stages present in the dry mud and their culturing in laboratory (Sars, 1885). Following the guidelines provided by Van Damme and Dumont (2010) and Marrone *et al.* (2019), sediment subsamples from each site were rehydrated at the University of Palermo using 4-litres plastic aquaria where a thin layer of sediment (about 5 mm) was introduced with 3 l of distilled water. Upon the first flooding of the aquaria, material sticking to the sides (potentially including crustacean resting stages) was rinsed down using a separate pipette for each aquarium. Distilled water was added from time to time to replace the water lost through evaporation.

Two culturing trials were run, namely between May and June 2019 and between May and July 2021, both under natural light and at room temperature between 25 and 28°C, and between 22 and 28.2°C, respectively. A feeble aeration was also provided.

Cultures were maintained for about 2-3 months and monitored each second day. Hatched crustaceans were collected monthly by filtering the water over a 125 µm plankton net. Moreover, some specimens belonging to poorly represented species in the cultures were individually collected by pipetting. All collected crustaceans were preserved in 90% ethanol.

Morphological and taxonomic analysis of ostracods

Both soft parts, dissected in glycerine and placed on sealed slides using nail polish, and valves, stored dry in micropaleontological slides (Namiotko *et al.*, 2011), were checked for species identification. Digital images of valves were acquired using a Zeiss EVO MA 10 scanning electron microscopy (SEM). Drawings of soft parts were made with the help of a camera lucida equipped microscope (Zeiss 47 30 11-9901). Chaetotaxy of soft parts follows Meisch (2000) and Scharf *et al.* (2020). All the figured specimens are adults. The ostracod classification and nomenclature used for taxonomic analysis follow Meisch *et al.* (2024).

The analysed material (preceded by the code "GR") is stored in the ostracod collection of the first author at the Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, unless differently specified.

DNA isolation, polymerase chain reaction and sequencing

Total genomic DNA was extracted starting from selected ostracod specimens using the BIORON GmbH "Ron's Tissue DNA Mini Kit", following the manufacturer's protocol. A single individual of *Heterocypris* sp. hatched from D3 lab culture, thus we kept it for morphological analyses, refraining from extracting its DNA. The selective amplification of a fragment from the gene encoding the Cytochrome c oxidase subunit 1 (COX1), was carried out by polymerase chain reaction (PCR) using the COX1 primers pair described by Folmer *et al.* (1994) and following the procedure described in Kvist *et al.* (2022).

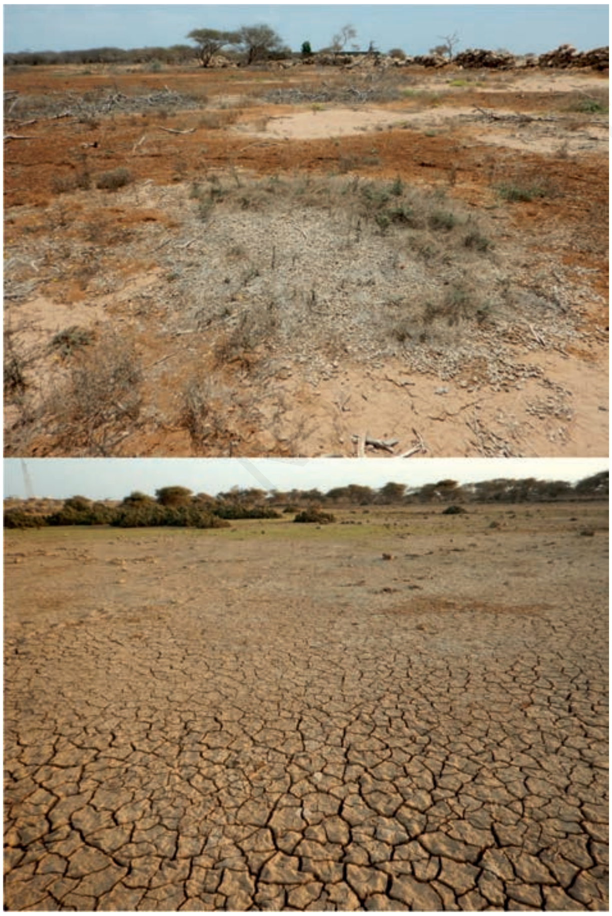

Fig. 1. Photographs of the ponds on Dahlak Kebir Island from which dry mud samples used for this study were collected: D2 (top) and D3 (bottom).

The PCR mix consisted of 19.8 µl of distilled water, 2.5 µl of Buffer 10X which includes 20-25 mM of $MgCl_2$, 0.3 µL of dNTPs (10 mM for each), 0.3 µl of each of the primers (10 µM), 0.3 µL of Taq polymerase (5 U µL) and 1.5 µL of template DNA, for a total volume of 25 µL. The thermal cycle consisted of 35 cycles of denaturation (95°C for 45 s), annealing (45°C for 45 s) and extension (72°C for 1 min), followed by 5 min at 72°C for the final extension step.

After PCR, 4 µL of each PCR product were used to perform electrophoresis on 1% agarose gel at 90 V for 25 min and then visualized with a UV transilluminator. When PCR products showed a clear single band, of the expected length, they were purified using the Exo-SAP-IT® kit (Affymetrix USB, Santa Clara, CA, USA). Sequencing was performed by Macrogen Europe (Milan, Italy - https://order.macrogen-europe.com/) through an ABI 3130×L sequencer (Applied Biosystems). The same primers used for the PCR were used for the direct sequencing of the PCR products. The software MEGA11 (Tamura *et al.*, 2021) was used for chromatogram analysis. Produced sequences were uploaded to the public database GenBank.

Abbreviations used in the text and figures

Cp, carapace; H, valve height; L, valve length; LV, eft valve; RV, right valve; iv, internal view; ev, external view; lv, lateral view; dv, dorsal view; vv, ventral view; cil, calcified inner lamella; il, inner list; im, inner margin; ucil, un-calcified inner lamella; A1, first antenna; A2, second antenna; RO, Rome organ; Md, mandibula; Mx1, maxillula; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod; CR, caudal ramus/rami; exo, exopodite on A2; ya, aesthetasc on A1; t_{1-4} and z_{1-3}, setae on A2; Y and y_{1-3}, aesthetascs on A2; G_{1-3}, GM, Gm, claws on A2; f, g and h_{1-3}, setae and claws on T2 and T3; d_{1-2} and d_p, setae on T3; S_a and S_p, anterior and posterior setae on CR; G_a and G_p, anterior and posterior claws on CR.

RESULTS

Based on the different salt contents of the collected soil samples, different electrical conductivity values were registered; these ranged from 1310 to 2180 µS cm^{-1} in D2, and from 1148 to 4500 µS cm^{-1} in D3.

A rich and diverse crustacean fauna hatched from both culturing attempts. Rather unexpectedly, no Copepoda hatched from any of the samples or culturing attempts. Branchiopoda are currently under study and will be the object of a separate publication. A total of five ostracod species were obtained, including two species new to science, as shown below.

Taxonomic account

Class Ostracoda Latreille, 1802
  Subclass Podocopa Sars, 1866
    Order Podocopida Sars, 1866
      Suborder Cypridocopina Baird, 1845
        Superfamily Cypridoidea Baird, 1845
          Family Cyprididae Baird, 1845
            Subfamily Cypridinae Baird, 1845
              Genus *Cypris* O.F. Müller, 1776

Cypris galefensis Martens, 1990
(Fig. 2)

Diagnosis of the species (modified after Martens, 1990)
♀: Cp very elongated, in dv pointed anteriorly and rounded posteriorly, with lateral sides running straight and parallel to each other over 1/4- l/3 of their total length; in vv, right anterior valve margin lip-like; in lv, left anterior selvage narrow; LV in both sexes posteriorly with a row of tubercles between selvage and

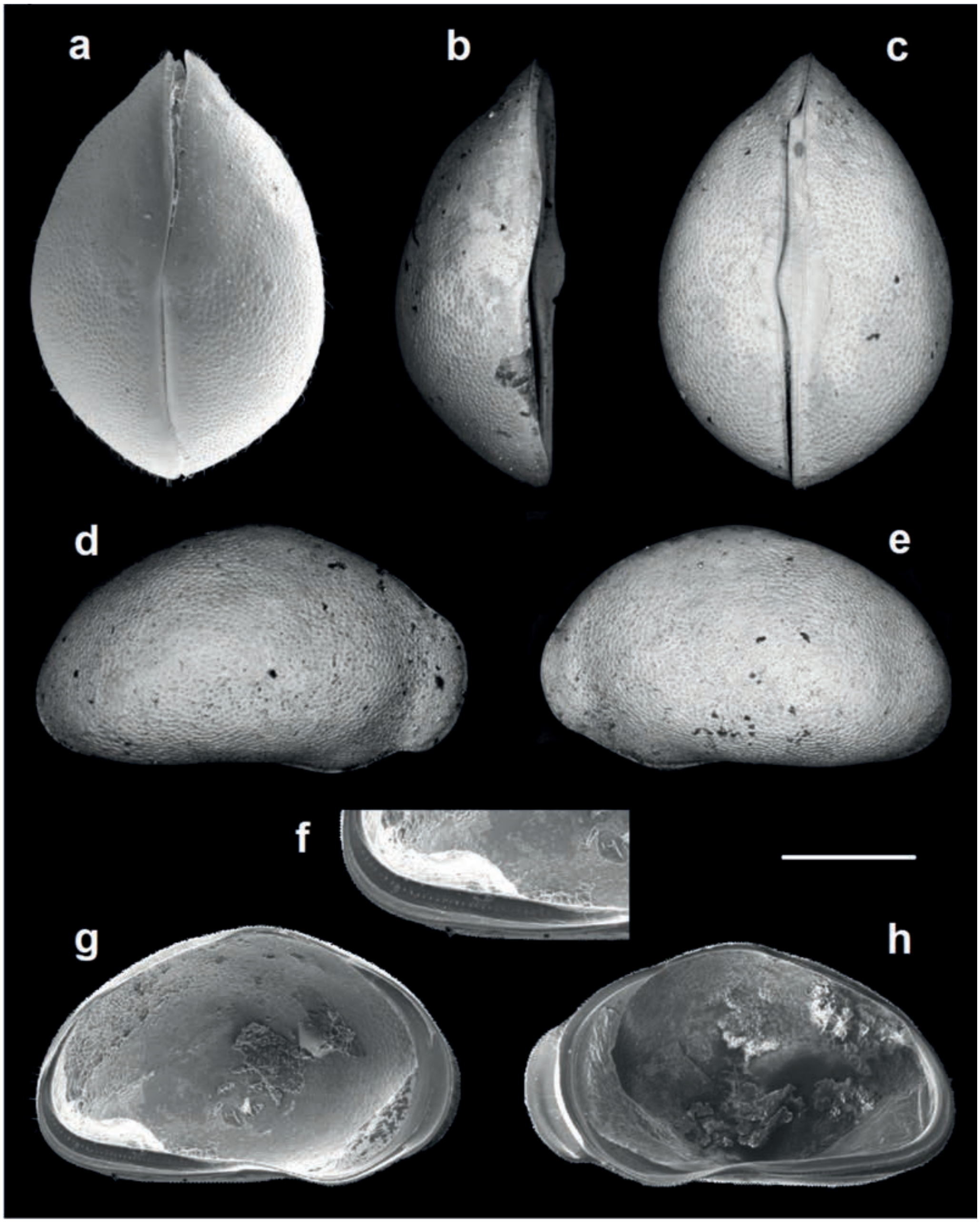

Fig. 2. *Cypris galefensis,* ♀. a) Cp dv (GR942); b) LV dv (GR828); c) Cp vv (GR943); d) RV ev (GR835); e) LV ev (GR835); f) LV iv, detail of postero- ventral corner showing the row of tubercles between selvage and inner list (GR944); g) LV iv (GR944); RV iv (GR835). Scale bars: a-e,g,h) 200 µm; f) 367 µm.

inner list. ♂: Cp more elongated than ♀, hemipenis with ventral lobe of lateral shield pointed, dorsal lobe small and narrow; medial shield asymmetrically produced towards the ventral side, with distal margin nearly straight; T1 palps narrow; left palp with terminal segment long and distally tapering, right palp with a squarish distal edge.

Material examined

Several specimens from D2 and D3 lab cultures, of which 9 dissected and/or used for SEM. All material is stored in the ostracod collection of the first author.
GenBank Accession Numbers: PQ538284 - PQ538285.

Measurements

L of ♀♀ (n=8): range 1615-1830 µm, mean±SD 1707.0±72.1 µm.

Remarks

The morphology of the valves and appendages (not figured) conforms to that of the type material illustrated by Martens (1990). No males were found in the analysed samples, but they were reported from the type locality of *Cypris galefensis*, a subterranean waterbody at Gal Ef, Somalia (approximate coordinates: 04°12’07’’N, 46°28’10’’E), which was the only other record available for this species to date.
The type material of *C. galefensis* was collected at Gal Ef (Somalia) by Giuseppe Messana and Lorenzo Chelazzi on 2 February 1982. The type material is deposited at La Specola Museum of Natural History in Florence, only the dissected holotype (♂, code MZUF523) and the dissected allotype (♀, code MZUF525) were found during a visit by IM and GR on 25 July 2024. The stored dry valves of the holotype are missing. Two paratypes are deposited in the Koninklijk Museum voor Midden-Afrika, (Tervuren, Belgium) and the remaining paratypes in the Koninklijk Belgisch Instituut voor Natuurwetenschappen, (Brussels, Belgium) (Martens, 1990).

Family Cyprididae Baird, 1845
Subfamily Cypridopsinae Kaufmann, 1900
Tribe Cypridopsini Kaufmann, 1900
Genus *Plesiocypridopsis* Rome, 1965

Plesiocypridopsis newtoni (Brady and Robertson, 1870)
(Figs. 3a and 4)

Diagnosis of the species (modified after Meisch, 2000)

♀: Cp markedly variable in length and shape, approximately reniform in lv, greatest H situated in front of mid-length; in dv, Cp anterior end slightly pointed, posterior end rounded; RV overlaps LV anteriorly, valves posteriorly about equally long; greatest W situated behind mid-length; selvage of RV anteriorly distinctly prominent; RV slightly longer than LV; A2 natatory setae extending beyond tips of terminal claws with ca 20% of their total length; T2 heavily built, terminal claw unusually short, strong and thick; seta h_3 small but always present. CR flagelliform, trunk cylindrical. ♂: Cp slightly smaller than that of ♀; terminal claws of A2 and T2 more heavily serrated; right T1 palp elongated, the left one short and stout; hemipenis with two distal lobes, the longer one finger-shaped, curved outwards and bearing a small knob-shaped process on its inner margin.

Material examined

Many specimens from D2 and D3 lab cultures, of which 10 dissected and/or used for SEM. All material is stored in the ostracod collection of the first author.
GenBank Accession Number: PQ538289.

L of ♂♂ (n=4): range 596-680 µm, mean±SD 639.0±47.4 µm;
L of ♀♀ (n=3): range 678-690 µm, mean±SD 696.0±21.6 µm.

Geographic distribution

This common species occurs in the Afrotropical, Oriental and Palaearctic regions (Meisch *et al.*, 2024).

Remarks

T1 palps of *Plesiocypridopsis newtoni* males show great variability in different populations, while the morphology of the hemipenes is considerably more constant, as reflected in the various drawings available for these characters in the literature (see Fig. 162 B-D in Meisch, 2000 and Fig. 139 g-i in Karanovic, 2010) and those of the specimens examined in the present study (Fig. 4).

Family Cyprididae Baird, 1845
Subfamily Cyprinotinae Bronstein, 1947
Genus *Heterocypris* Claus, 1892

Diagnosis of the genus (modified from Meisch, 2000)

♀: Cp with variable H/L ratio; anterior and posterior margins usually approximately similarly broadly rounded; LV slightly overlaps RV at both ends; outer marginal zone of RV anteriorly and posteriorly with a row of pustules variable in number and shape, sometimes weakly expressed or totally missing; terminal palp segment of maxillula cylindrical, both teeth bristles of third masticatory lobe distinctly serrated; T2 seta d_2 missing, d_1 short, h_1 unusually long; CR weakly curved, terminal claws long, antero-distal seta about half the length of the long terminal claw, posterior seta conspicuously long, ca 4/5 the length of the short terminal claw, posterior margin with a row of fine setulae. ♂: Cp similar to that of the ♀ but slightly shorter; CR distinctly curved.

***Heterocypris* sp.** (Ramdohr, 1808)
(Fig. 3b)

Material examined

One adult ♀ (GR953, 1150 µm), the only one hatched from D3 lab culture, dissected and RV valve used for SEM (LV damaged).

Remarks

The morphology of soft parts (not illustrated) of the analysed ♀ specimen is consistent with those of the genus. The surface of the valves is smooth in external view, margin triangular in lateral view. RV smaller than LV. Maximum length below mid-height, maximum height slightly posterior. Posterior and anterior ends evenly rounded. In iv (Fig. 3b), ventral margin sinuous, with a central concavity and an anterior subtle convexity. Posteroventral outer marginal zone of RV characterised by the typical row of pustules.

In view of the high plasticity in the valve morphological features in this genus (Martens *et al.*, 2002), it was considered advisable to make the identification to genus level, and to provide a more detailed description should further specimens become available in the future.

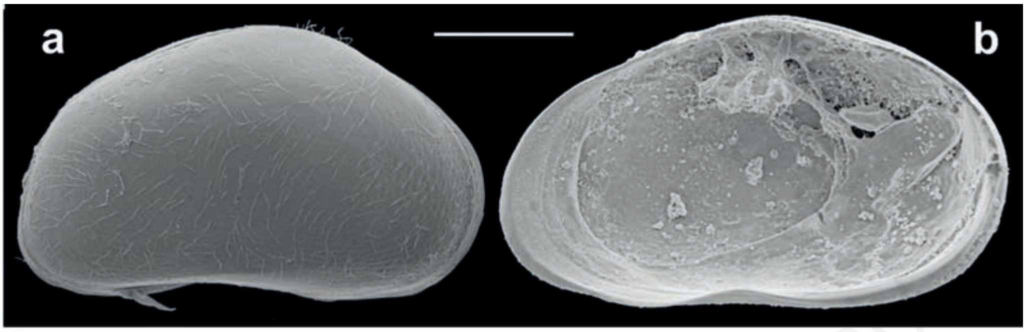

Fig. 3. a) *Plesiocypridospsis newtoni*, ♀, Cp right lv (GR981); b) *Heterocypris* sp., ♀, RV iv (GR953). Scale bars: a) 200 µm; b) 285 µm.

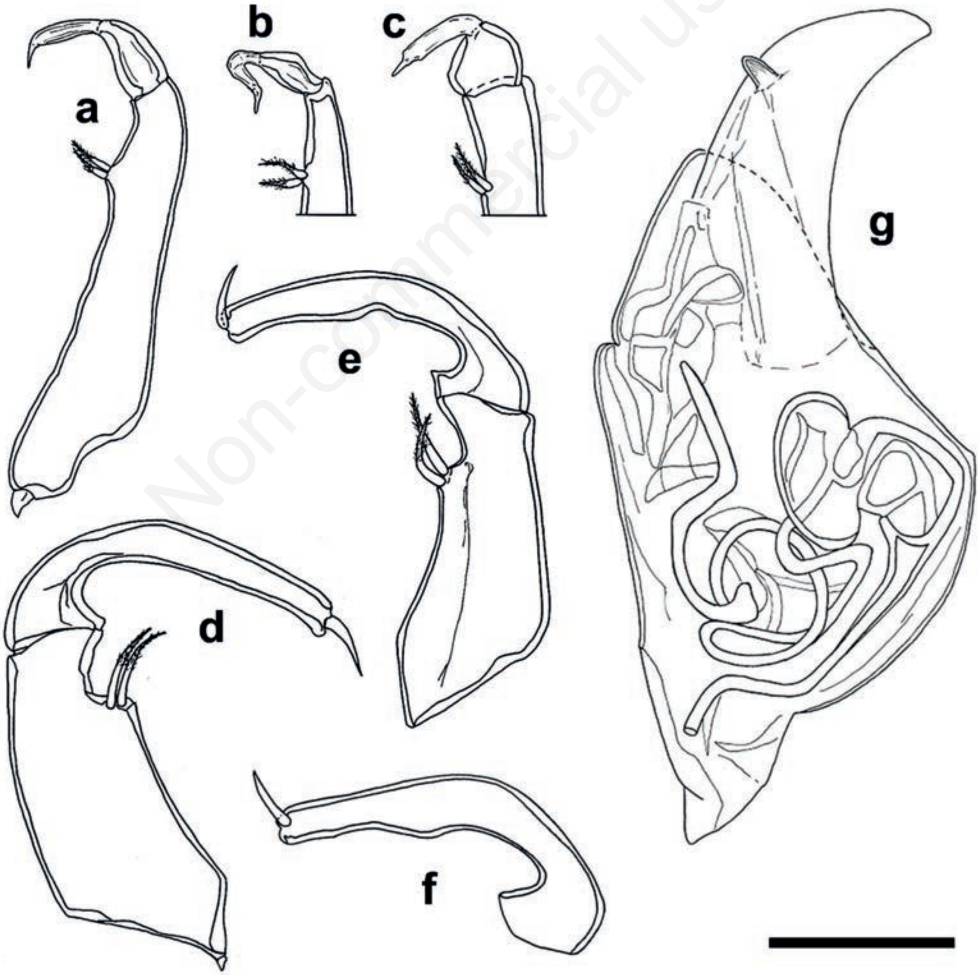

Fig. 4. *Plesiocypridopsis newtoni*, ♂. a) T1 left palp (GR962); b) T1 left palp (GR964); c) T1 left palp (GR963); d) T1 right palp (GR962); e) T1 right palp (GR964); f) T1 right palp (GR963); g) hemipenis (GR963). Scale bar: 50 µm.

Family Cyprididae Baird, 1845
Subfamily Herpetocypridinae Kaufmann, 1900
Genus *Chrissia* Hartmann, 1957

Diagnosis of the genus (from Savatenalinton, 2023)
Cp medium to large (L c. 1.5-3.5 mm), elongated or reniform shaped and laterally compressed; RV and LV without selvages; anterior calcified inner lamella well-developed, with or without inner list, inner list (if present) situated very close to valve margin; marginal septa absent; fused zone wide, pore canals straight along anterior and posterior margins, branched along ventral margin, false pore canals present, especially ventrally; RO on A1 small, not segmented; natatory setae on A2 of medium length (reaching not beyond tip of end claws); terminal segment of Mx1 palp rectangular and elongated (L c. 2-3 times of W); setae d_1 and d_2 on T2 short, subequal; CR asymmetrical, S_p seta absent, spines on ventral margin of right ramus larger than on left ramus, sometimes very long and stout; hemipenis without hook-like process on medial shield, up to two additional internal coils of spermiduct.

Chrissia martensi n.sp.
(Figs. 5-7)

https://www.zoobank.org/NomenclaturalActs/70bc621f-ac0b-46c4-9a92-0be03927709a

Note: authorship of *Chrissia martensi* n.sp. is attributed to GR and IM and should be cited as "Rossetti and Mazzini" in "Rossetti *et al.* 2025" (ICZN 2000, Recommendation 51E).

Type locality
Pond D3, Dahlak Kebir Island, Dahlak Archipelago, Eritrea, lat. 15.66986 N, long. 39.97079 E (Fig. 1).

Type material
Holotype: ♀ (GR971-MZUF696, from D3 lab culture), soft parts dissected in glycerine in a sealed slide, valves stored dry in a micropaleontological slide. Paratypes: one ♀ (GR827-MZUF697, from D3 lab culture), soft parts dissected in glycerine in a sealed

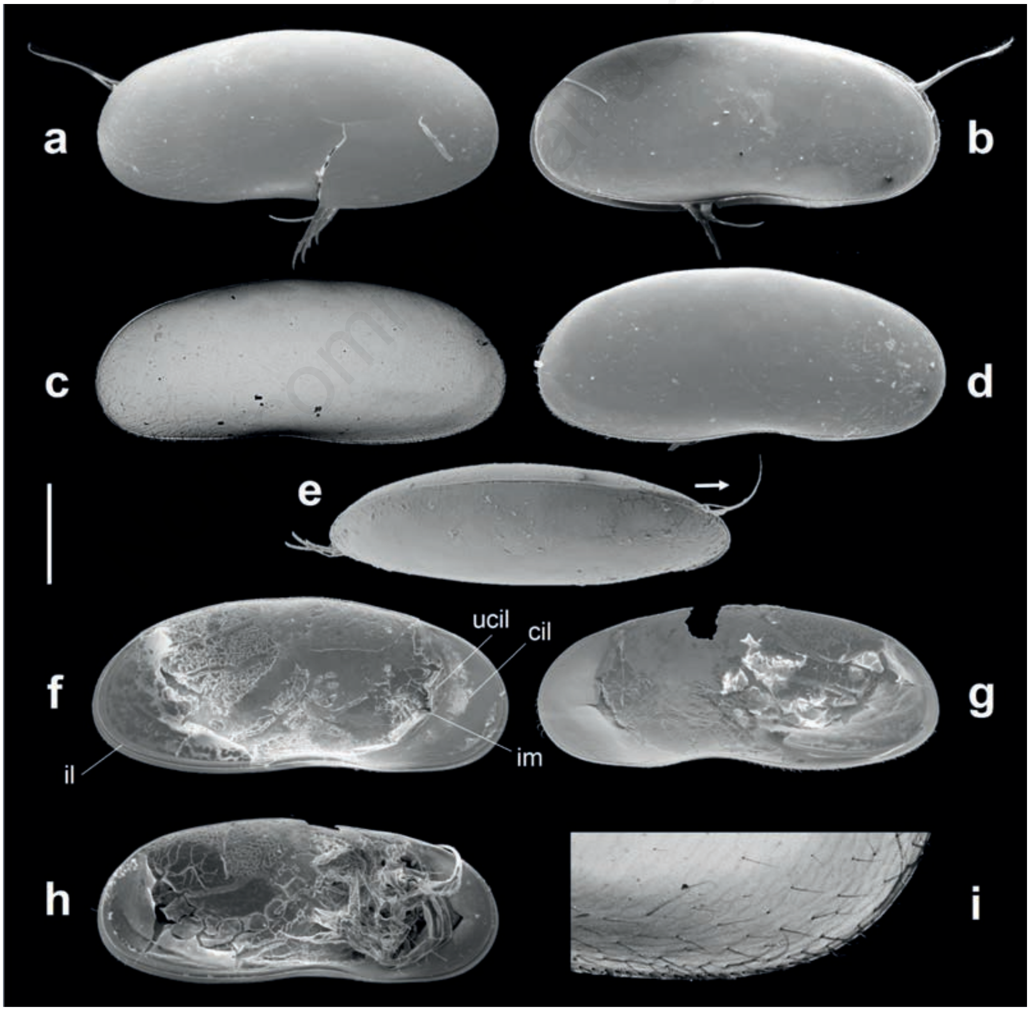

Fig. 5. *Chrissia martensi* n.sp., ♀. a) Cp L ev (GR941); b) Cp right ev (GR941); c) Cp left ev (GR827-MZUF697); d) Cp right ev (GR834); e) Cp dv (GR983); f) LV iv (GR834); g) RV iv (GR968); h) LV iv (GR969); i) detail postero- ventral corner of Cp (GR827-MZUF697). Scale bars: a-h) 500 µm; i) 250 µm.

slide, valves used for SEM; two adult ♀♀ from D3 stored *in toto* in ethanol (no numbers). Additional material: one ♀ (GR950-MZUF698, from D2 lab culture), soft parts dissected in glycerine in a sealed slide, valves stored dry in a micropaleontological slide. Type and additional material are deposited in the crustacean collection of La Specola Museum of Natural History, Zoology Section Florence, Italy; the number after the acronym MZUF indicates the collection number of the deposited specimens.

Other material examined

Many specimens from D2 and D3 lab cultures, of which 9 dissected and/or used for SEM. All material is stored in the ostracod collection of the first author.
GenBank Accession Number: PQ538286.

Measurements

L of ♀♀ (n=11): range 1915-2060 µm, mean±SD 1982.0±46.2 µm.

Diagnosis

♀: Cp elongated and laterally compressed; H/L c. 40%; postero-ventral margin of valves rounded, no protusions or spines present; outer surface of valves covered with sparse setae; RO on A1 small, unsegmented; A2 with aesthetasc Y three-segmented; natatory setae not reaching tips of terminal claws; additional setae on distal segment reduced to spines; terminal segment of Mx1 palp cylindrical, L c. 2.2 times the W; T2 with d-setae present; T3 with pincer organ; h_1 seta very small; h_3 seta slender in the distal half; CR asymmetrical and slender, S_p setae absent, G_a and G_p with spines of different length; ventral margin with spines, much finer on left ramus. ♂ unknown.

Description of adult ♀

Cp and valves

Carapace elongated, with its greatest height in the posterior half (Fig. 5 a-d). Valves fragile and thin. Anterior and posterior margins rounded. Ventral margin with convexity slightly anterior. Dorsal margin gently arched, tapering anteriorly, the LV slightly overlapping the RV at the anterior edge of the dorsal margin (Fig. 5e). Surface generally smooth with normal pore canals (*sensu* Puri and Dikau 1969) and sparse setae. In some valves, a reticulate pattern of striations has been observed (Fig. 5 c,i) in the posterior area. The lines of that reticulation consist of inconspicuous, linear, small shallow pits arranged in a sort of reticulation that becomes fainter in the central and anterior part of the valves. In iv, marginal septa absent; calcified inner lamella wider anteriorly (Fig. 5 f,g). The inner margin runs between the shell edge and the fused zone. The fused zone at the anterior, ventral, and posterior margins is approximately the same width (Fig. 5h). Marginal pore canals simple and fine, most of them open on the shell surface. LV overlaps RV ventrally.

Soft parts

A1 (Fig. 6a): seven-segmented, first segment with one long dorsal seta and two long ventral setae; second segment with small RO ventrally; third segment with a short dorso-apical seta; fourth segment with two ventral-apical setae, one short and one reaching tip of sixth segment, and two long dorsal setae; fifth segment with four log apical setae, two dorsal and two ventral; sixth segment with two long dorsal setae and three ventral setae, two long and one slightly exceeding tip of last segment; seventh segment with four long setae and one short dorsal seta (reaching tip of terminal segment). Terminal segment apically bearing three setae, two long and one much shorter, and an aesthetasc y_a c. twice as long as the shortest seta. A2 (Fig. 6b): protopodite with three proximal ventral setae, two relatively long and sub-equal, one very short, and a distal ventral seta c. as long as the first endopodal segment; exopodite with three setae, one long and two short; first endopodal segment with a tripartite aesthetasc Y at about one third of the ventral margin, a ventro-apical seta slightly longer than half of the next segment, and in dorso-apical position five natatory setae not reaching the tip of terminal claws and a short seta more externally; second endopodal segment undivided, with setae t_{1-4} of different lengths midway along ventral margin and slightly further back along dorsal margin two subequal setae exceeding distal margin of segment itself, apically one aesthetasc y_2 about one-third as long as next segment and three serrated claws G_{1-3}, of which G_1 and G_3 are of similar length and G_2 c. 3/4 of the former ones, and three setae z_{1-3} subequal and reaching approximately to the tip of the terminal claws; terminal endopodal segment apically bearing two terminal claws of which G_m is about 3/5 of G_M, a seta g of similar length of G_m, an aesthetasc y_3 with accompanying seta barely longer than the latter, and a robust spine in ventral position. Md-coxa (Fig. 6c): slender, masticatory part with approximately eight teeth, with one antero-subapical setae and two smaller postero-apical setae. Mx1 (Fig. 7a): palp with two segments, basal one with five long unequal ventro-apical setae longer than the next segment and a shorter dorsal subapical seta, terminal segment with six claws. T1 (Fig. 7b): palp with three apical setae, the central one about twice as long as the lateral ones. T2 (Fig. 7c): basal segment with seta d_1 small, about 1/3 the length of d_2; second segment with seta e reaching mid-length of next segment; penultimate segment subdivided, proximal part with seta f of its same length, distal part bearing a seta g about 3.5 times as long as the last segment; terminal segment apically with two short lateral setae (h_1 about half the length of h_2) and a robust claw about as long as the last and penultimate segments together and with serrulated distal half. T3 (Fig. 7d): first segment with long setae d_1, d_2 and d_p, the latter slightly longer than the previous two; second segment with long apical seta e; third segment undivided, with seta f inserted in median position and reaching just beyond the tip of the terminal segment; last segment with pincer organ, tiny seta h_1, curved seta h_2 and long seta h_3, the latter slightly shorter than the third segment and approximately twice as long as h_2. CR attachment (Fig. 7e): triangular basal loop, dorsal branch short and straight with curved end, ventral branch longer and with distinctly arched initial part. CR well-developed and asymmetrical. Right CR (Fig. 7f): ventral margin with spines in distal 3/5, initially finer and progressively more robust; claw G_p with spines of different length and more expressed in the first half; claw G_a about 11/7 as long as G_p, with spines in distal 2/5 and finer at its extremity; seta S_a of similar length as G_p, seta S_p absent. Left CR (Fig. 7g): very fine spines in the distal 2/5 of the ventral margin; seta S_a and claw G_p of equal length and about 7/12 as long as G_a; both terminal claws with spines of unequal length and weaker than in right CR; seta S_p absent.
Male unknown.

Differential diagnosis

Taking into account the matrix by Kong *et al.* (2014), supplemented with the characters of the other species of the genus *Chrissia* considered as valid by Meisch *et al.* (2024), *Chrissia martensi* n.sp. exhibits a combination of valve and soft part characters not found in any other representative of the genus.

Etymology

The species is named after Koen Martens as a humble tribute to a great scientist who made an enormous contribution to the study of the diversity of non-marine ostracods, and in particular African ostracod faunas.

Distribution

Chrissia martensi n.sp. is so far known from the pond D3 (type locality) and the neighbouring pond D2 on Dahlak Kebir Island, where dry mud was collected that once rewetted generated the specimens described here.

Remarks

In the individuals examined, the ventro-apical seta on the first endopodal segment of A2 was either totally missing, present on only one of the two antennae, or present on both. This could indicate either intraspecific variability for this character, or a marked fragility of this seta that tends to detach easily during dissection.

No sperm were found inside the carapace of females, which further confirms the absence of males in the material studied.

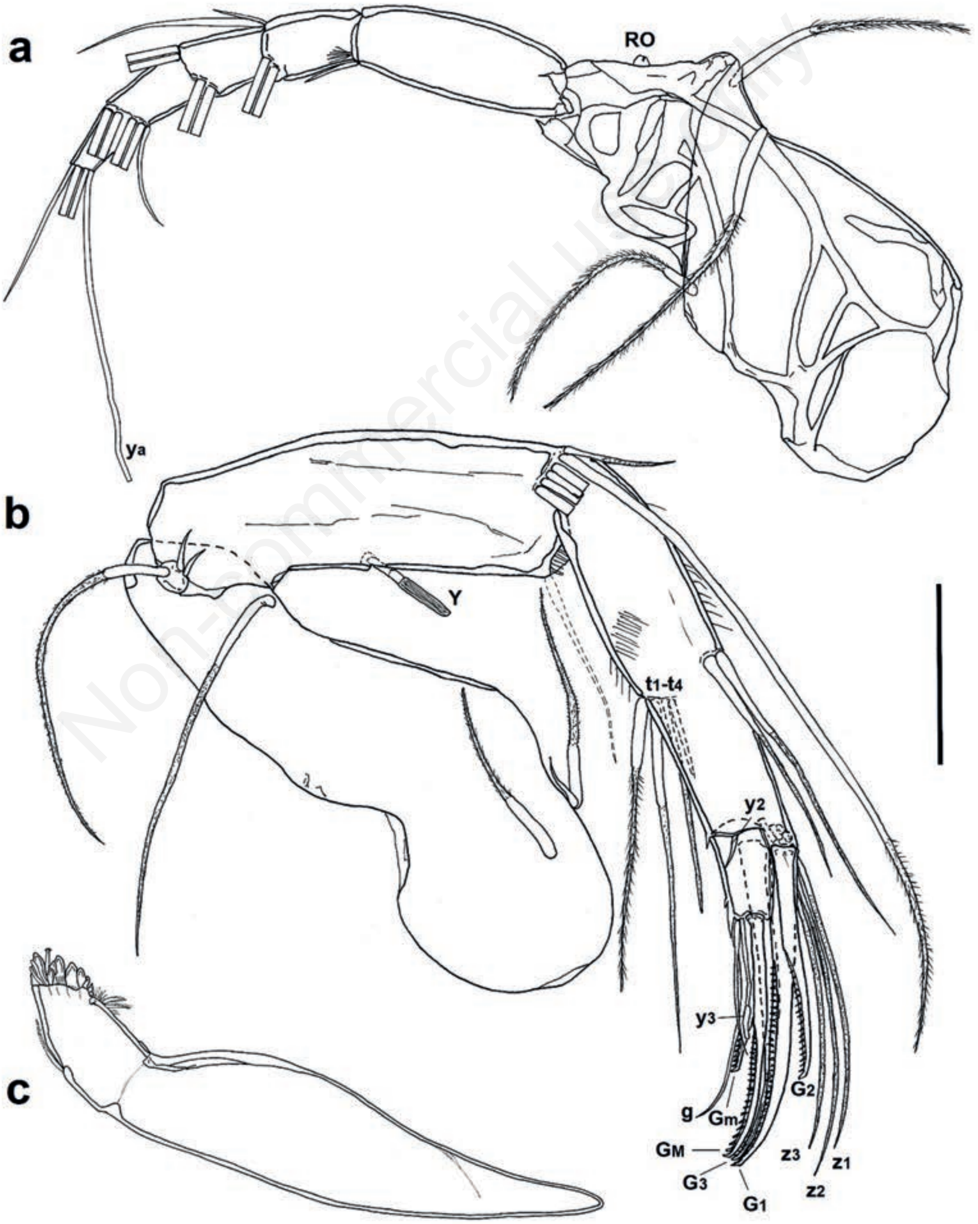

Fig. 6. *Chrissia martensi* n.sp., ♀ (GR827-MZUF697). a) A1; b) A2; c) Md-coxa. Scale bar: 100 µm.

Family Ilyocyprididae Kaufmann, 1900
Subfamily Ilyocypridinae Kaufmann, 1900
Genus *Ilyocypris* Brady and Norman, 1889

Diagnosis of the genus (modified from Karanovic, 2012 and Mazzini *et al.*, 2014)

Subquadrate valves, not highly asymmetrical; LV overlapping RV on all sides; postero-lateral depressions, lateral tubercles, marginal spines and anterior ridge present or absent; calcified inner lamella very narrow; natatory setae on both A1 and A2 usually long, sometimes reduced on A2; Mxl palp stout, with first segment distally not distinctly dilated, and second segment short and broad; T1-palp in ♀ divided, in ♂ prehensile palps asymmetrical or symmetrical; T2 four- or five-segmented; hemipenis with inner, medial and outer lobes.

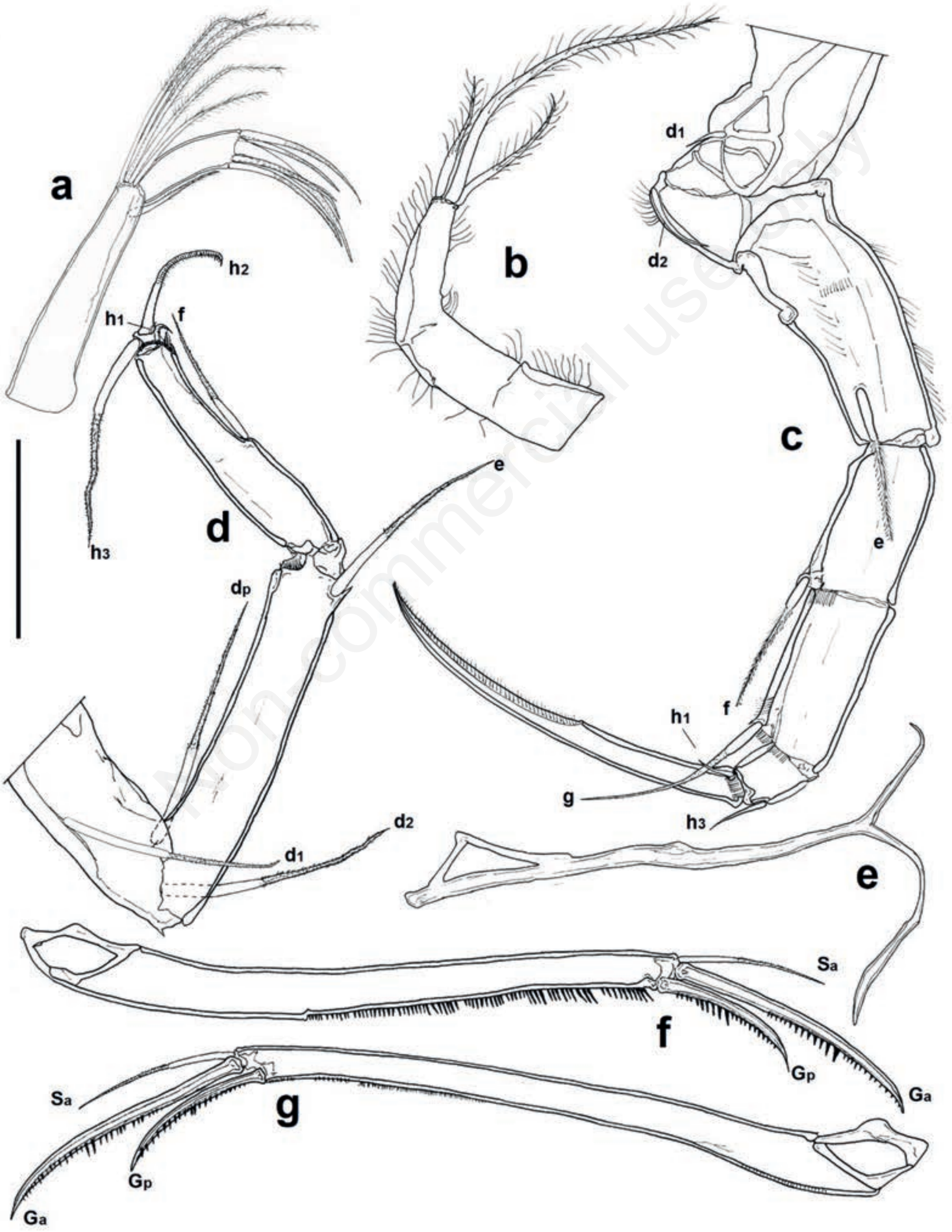

Fig. 7. *Chrissia martensi* n.sp., ♀ (GR827-MZUF697). a) Mx1 palp; b) T1 palp; c) T2; d) T3; e) CR attachment; f) right CR; g) left CR. Scale bars: a,e) 250 μm; b) 125 μm; c,f,g) 200 μm; d) 138 μm.

Ilyocypris dahlakensis n.sp.
(Figs. 8-13)

https://www.zoobank.org/NomenclaturalActs/af3814ca-a560-48b4-a10a-e2ee60e14746

Note: authorship of *Ilyocypris dahlakensis* n.sp. is attributed to GR and IM and should be cited as "Rossetti and Mazzini” in “Rossetti *et al.* 2025" (ICZN 2000, Recommendation 51E).

Type locality

Pond D2, Dahlak Kebir Island, Dahlak Archipelago, Eritrea, lat. 15.67046 N, long. 39.97075 E (Fig. 1).

Type material

Holotype: ♂ (GR954-MZUF699, from D2 lab culture), soft parts dissected in glycerine in a sealed slide, valves stored dry in a micropaleontological slide. Paratypes: one ♂ (GR959-MZUF700, from D2 lab culture) and three ♀♀ (GR955-MZUF701, GR956-MZUF702 and GR960-MZUF703, all from D2 lab culture) with soft parts and valves as the holotype; two adult ♂♂ and two adult ♀♀ from D2 lab culture stored *in toto* in ethanol (no numbers). Additional material: one ♂ (GR826-MZUF704, from D3 lab culture), soft parts dissected in glycerine in a sealed slide, valves used for SEM. Type and additional material are deposited in the crustacean collection of La Specola Museum of Natural History, Zoology Section Florence, Italy; the number after the acronym MZUF indicates the collection number of the deposited specimens.

Other material examined

Many specimens from D2 and D3 lab cultures, of which 9 dissected and/or used for SEM. All material is stored in the ostracod collection of the first author.
GenBank Accession Numbers: PQ538287- PQ538288.

Measurements

L of ♂♂ (n=9): range 520-558 µm, average±SD 546.0±12.1 µm; L of ♀♀ (n=4): range 543-585 µm, average±SD 569.5.0±18.3 µm.

Diagnosis

Small-sized *Ilyocypris* with sulci, and pits typical of genus. Peculiar sexual dimorphism, with ♀ valves displaying a straight posterior margin, forming a right angle at the ventral anterior edge. A2 natatory setae extending sligthly beyond tip of teminal claws; penultimate segment of cleaning leg with setae f and g; Mx-clasping organs nearly symmetrical; penultimate segment of walking leg undivided; Zenker organ with c. 21 rings of chitinous spines; hemipenis with straight medial margin and evenly rounded lateral

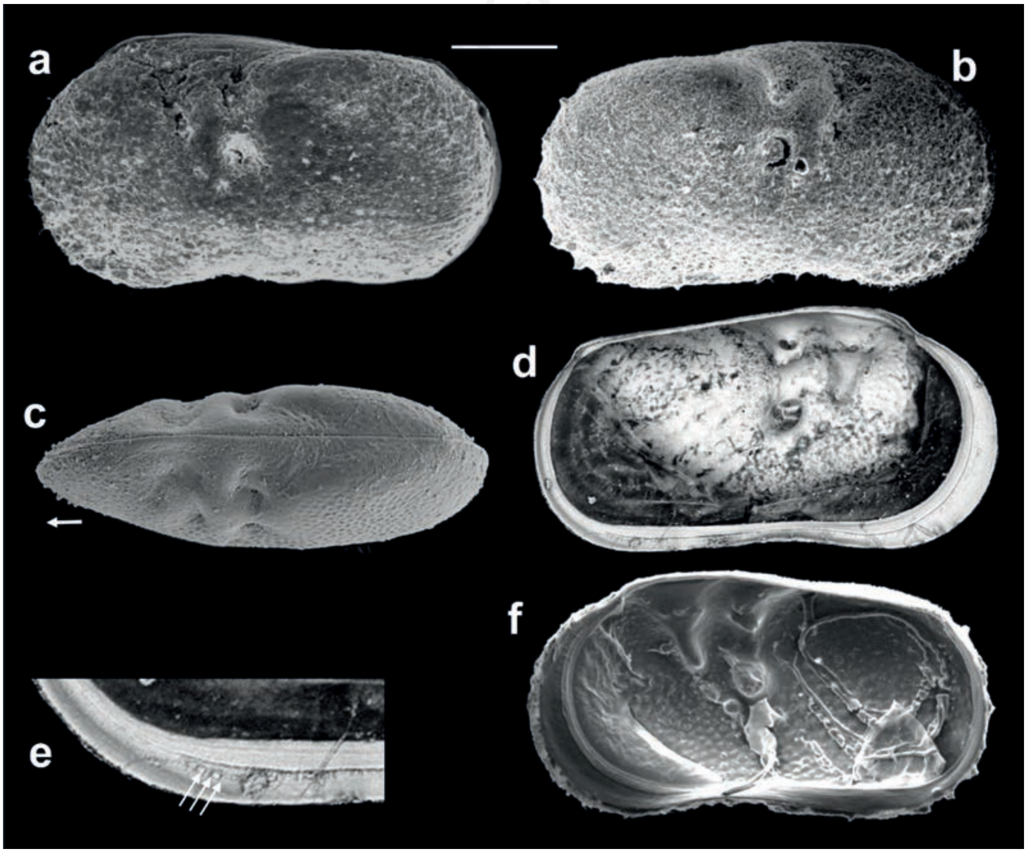

Fig. 8. *Ilyocypris dahlakensis* n.sp., ♂ (GR826-MZUF704). a) LV ev; b) RV ev; c) Cp, dv (GR984); d) LV iv; e) LV, detail postero-ventral margin with ripplets (indicated by arrows); f) RV iv. Scale bar: 200 µm.

margin, medial lobe (h) sub-squared, slightly longer or as long as inner lobe (b), the latter digitiform either straight or with a bump at the middle of its length and the distal end hook-like or rounded, outer lobe (a) short and stout and with outer-distal part usually rounded; uropodal ramus curved and enlarged in the proximal part, posterior seta reaching or slightly exceeding the base of the terminal claws, the latter subequal in length.

Description of adult ♂

Cp and valves

Shell sub-renal in lateral view (Fig. 8 a,b), with greatest height (antero-dorsal corner) at anterior third, anterior end roundly pointed, posterior end rounded. Dorsal margin slightly tapering anteriorly, with a blunt turn immediately behind the posterior sulcus due to inflation of the postero-dorsal margin. Ventral margin concave. Valves covered with round pits. Tiny spines/tubercles on the anterior, more marked on the RV (Fig. 8b). In dorsal view, marked dorsomedian sulci, LV overlapping RV posteriorly and dorsally, shell very narrow and without lateral alae (Fig. 8c). Calcified inner lamella relatively wide (Fig. 8 d,f) on both ends, but with anterior one slightly wider. On the anterior calcified inner lamella, three inner lists: from the interior, the first one is usually weakly expressed, sometimes not preserved; the second and third ones run close to the inner margin. The inner lists occur also on posterior calcified inner lamella, but the first one is very faint or absent. One row of fine, densely arranged marginal ripplets present on both anterior and posterior calcified inner lamellae. On the ventral posterior margin of LV two tubercles occur (Fig. 8e) that correspond to a single tubercle in the same position on the RV (Fig. 8f) with the possible function of additional closing mechanism of the valves. Muscle scars as for the genus.

Soft parts

A1 (Fig. 10a): seven-segmented; first segment with one long dorsal seta and two long, subequal ventral setae; second segment with a dorso-apical seta slightly exceeding tip of next segment; third segment with two apical setae, the ventral one about 2/3 of the dorsal one; fourth segment bearing two very long dorsal setae and two ventral setae in a length ratio of about 1:5 between them; fifth segment with two apical setae, the smaller of which barely exceeds the tip of the last segment, and two very long dorsal setae; penultimate segment apically with four long setae, two dorsal and two ventral, and a dorsal α seta about 3 5 times as long as the next segment; distal part of the seventh segment with three setae, one long and two smaller ones, of which the ventral one rather stout, and a shorter y_a aesthetasc. A2 (Fig. 10b): protopodite with three ventral setae, two short and one sub-apical longer, the latter reaching beyond the middle of the second endopodal segment; exopodite with two tiny setae and one long seta reaching beyond the distal margin of the first endopodal segment; first segment of endopodite with

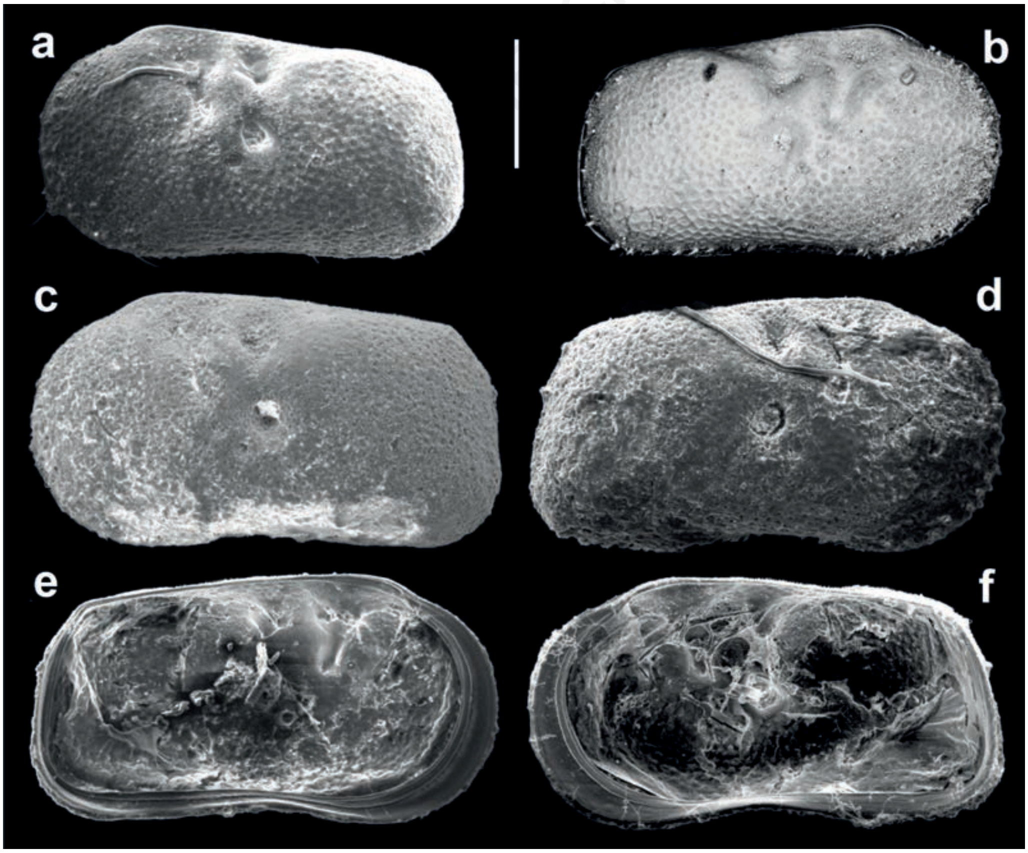

Fig. 9. *Ilyocypris dahlakensis* n.sp., ♀. a) LV ev (GR940); b) RV ev (GR940); c) LV ev (GR833); d) RV ev (GR833); e) LV iv (GR833); f) RV iv (GR833). Scale bar: 200 µm.

exopodite Y three-segmented along ventral margin, apically a ventral seta reaching beyond the tip of the last segment of endopodite, five natatory setae distinctly longer than the tip of the terminal claws, and more dorsally a seta slightly longer than the ventral seta; next segment undivided, proximally on ventral margin aesthetasc y_1 very short, setae t_{1-4} of different length (the longest exceeding distal margin of last segment), two setae one about half as long as the other in intermediate position along dorsal margin, sub-apically long setae z_{1-3} (z_1 claw-like) ending near tips of terminal claws or slightly longer, and apically two robust G_1 and G_2 claws, and G_3 claw-like just exceeding the last endopodal segment; terminal segment of the endopodite with G_M and G_m claws, of which the former a little longer, an aesthetasc y_3 slightly larger than y_1 and y_2 and its accompanying seta twice as long as the last segment. T1 (Fig. 10 c,d): endopodites in the form of almost symmetrical, two-segmented prehensile palps; proximal segment with two apical setae, distal segment slenderer and more sinuous, with a subapical seta slightly shorter than the distal tip. T2 (Fig. 11a): four segmented (penultimate segment undivided); first segment with seta d_1, seta d_2 absent; second segment with short apical seta e; penultimate segment undivided, with short, subequal setae f and g, the first inserted at about 1/3 the length of the segment and the second apically; terminal segment with short setae h_1 and h_3 in a ratio of approximately 2:1, h_2 a robust, finely serrated claw. T3 (Fig. 11c): first segment with seta d_1; second segment with apical seta e extending to 2/3 of next segment; third segment with medially seta f almost attaining tip of segment and long subapical seta g nearly reaching end of seta h_2, distal margin with spines; terminal segment with long, subequal h_1 and h_3 setae about twice as long as h_2. CR (Fig. 11c): slender and symmetrical; S_p inserted about 2/3 of the margin of ramus and 8/5 the length of S_a; G_a and G_p sub-equal and slightly longer than half of the length of ramus. Hemipenis (Figs.

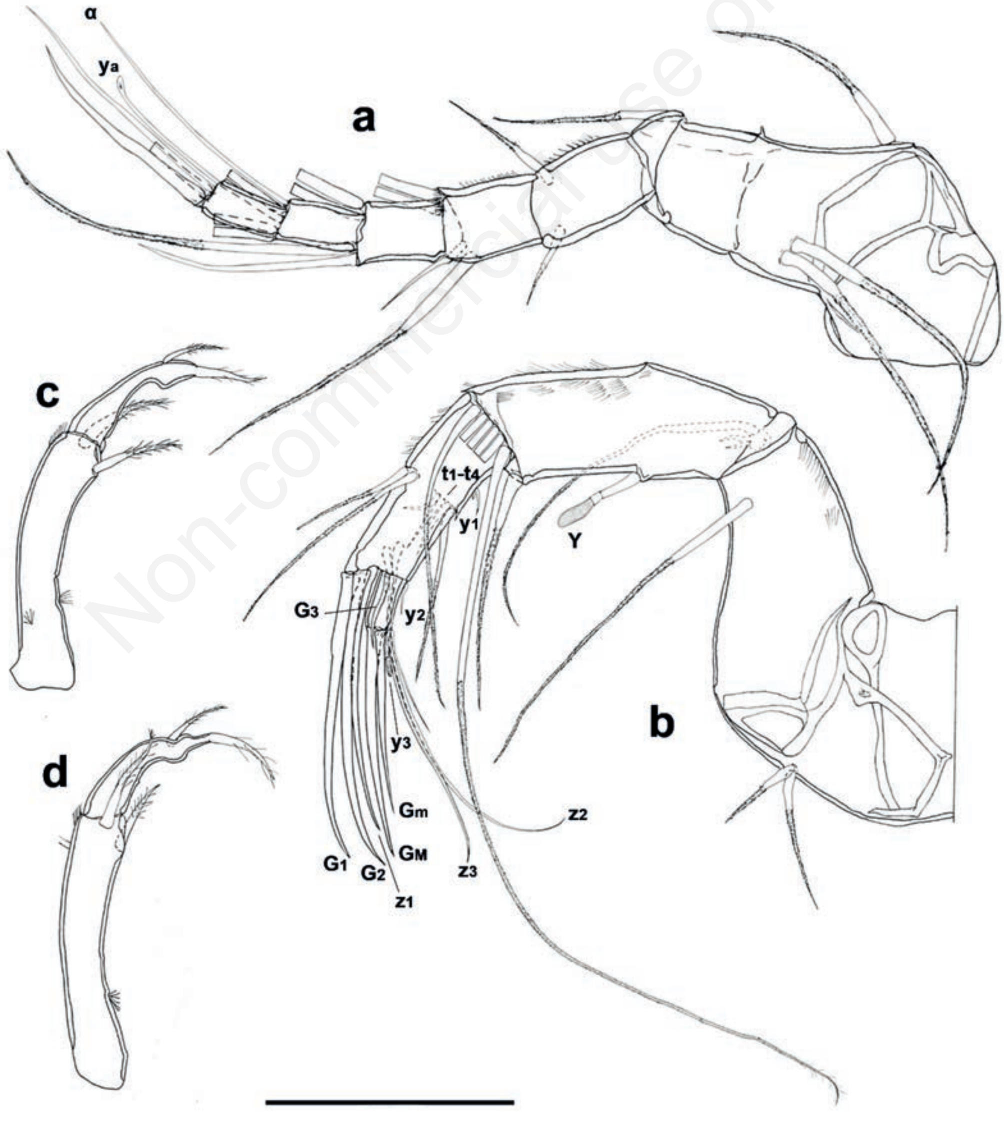

Fig.10. *Ilyocypris dahlakensis* n.sp., ♂ (GR826-MZUF704). a) A1; b) A2; c,d) T1-palps. Scale bar: 100 µm.

11 d,e and 12): inner margin straight, outer margin evenly rounded; outer lobe short and stout, partially overlapping middle lobe, usually narrower in its middle part, distal part subquadrate or with slightly rounded outer corner; middle lobe elongated, more expanded in its terminal part, distal edge subquadrate with rounded corners; inner lobe thin and long, reaching the tip of the middle lobe, with variable shape even in the same individual as regards outer margin (more or less sinuous) and distal end (circular to hook-like); copulatory process thin and distally curved. Zenker organ (Fig. 11f): slightly more expanded in its central part, with about 22 spiny whorls.

Description of adult ♀

Cp and valves

Shell sub-rectangular in lateral view (Fig. 9 a-d). Dorsal margin straight, sloping towards posterior end. Greatest H at the anterior cardinal angle. Anterior margin broadly rounded, posterior margin oblique with posterior cardinal angle >45° and a posterior ventral angle <45°. Ventral margin slightly concave near middle. Surface densely covered by small polygonal pits, with those in front of anterior sulcus, between two sulci and behind posterior sulcus, shallower (Fig. 9 a,b). Tiny spines around the exterior margin of RV, at the anterior margin of LV in some valves (Fig. 9 b-d). Dorsal sulci strongly marked, wider towards the dorsal margin. In internal view, calcified inner lamella relatively wide (Fig. 9 e,f), with the three lists evident anteriorly but almost fused at the ventral posterior angle. Marginal ripplets not visible on both valves. Muscle scars as for the genus.

Soft parts

All limbs as in ♂, except for last two segments of A2 and T1 (Fig. 13). A2 (Fig. 13a): penultimate segment with subequal claws G_{1-3}, setae z_{1-3} long, z_1 and z_2 almost reaching the tip of the terminal claws and z_3 slightly exceeding them. T1 (Fig. 13b): protopodite bearing two short setae a and more distally c. 16 setae of different length; endopodite a two-segmented palp, distal segment with three apical setae, of which the central one markedly longer; branchial plate with six hirsute rays.

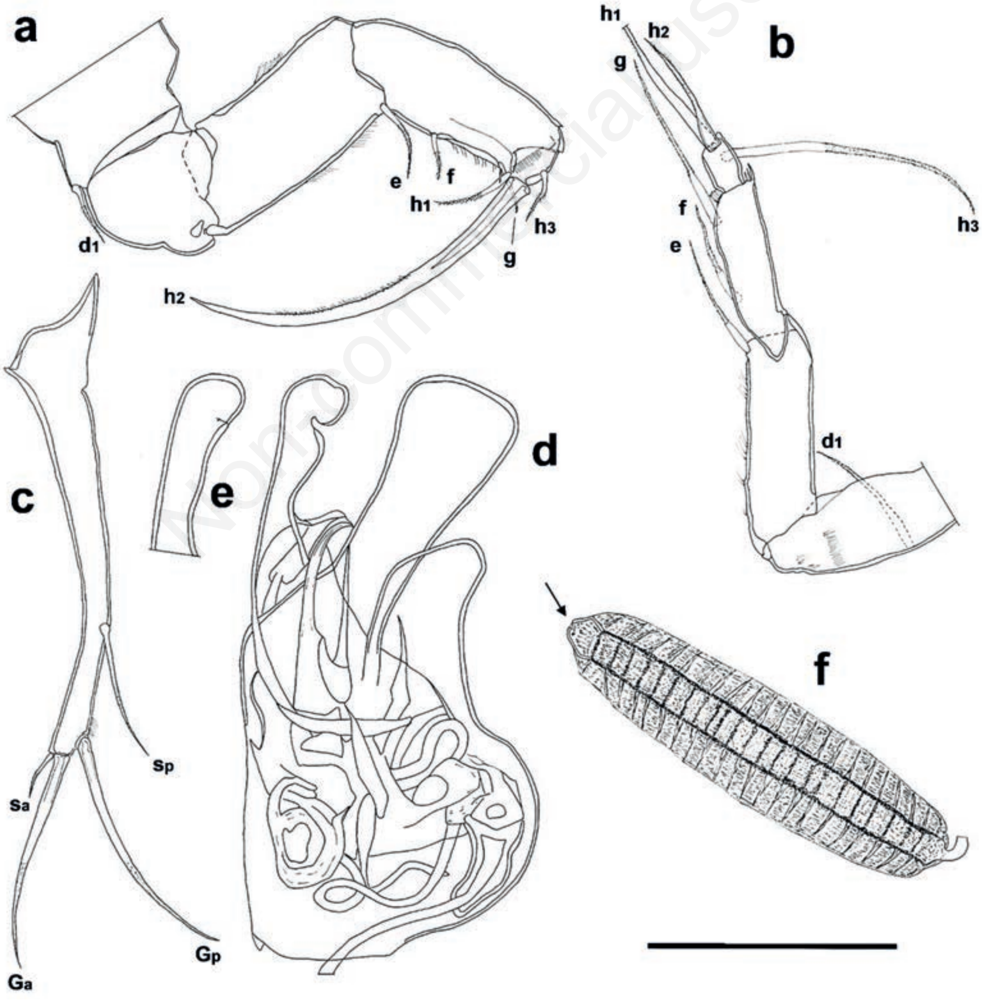

Fig. 11. *Ilyocypris dahlakensis* n.sp., ♂ (GR826-MZUF704). a) T2; b) T3; c) CR; d) hemipenis; e) inner lobe; f) Zenker organ (arrows indicates proximal end). Scale bars: a-e) 100 µm; f) 200 µm.

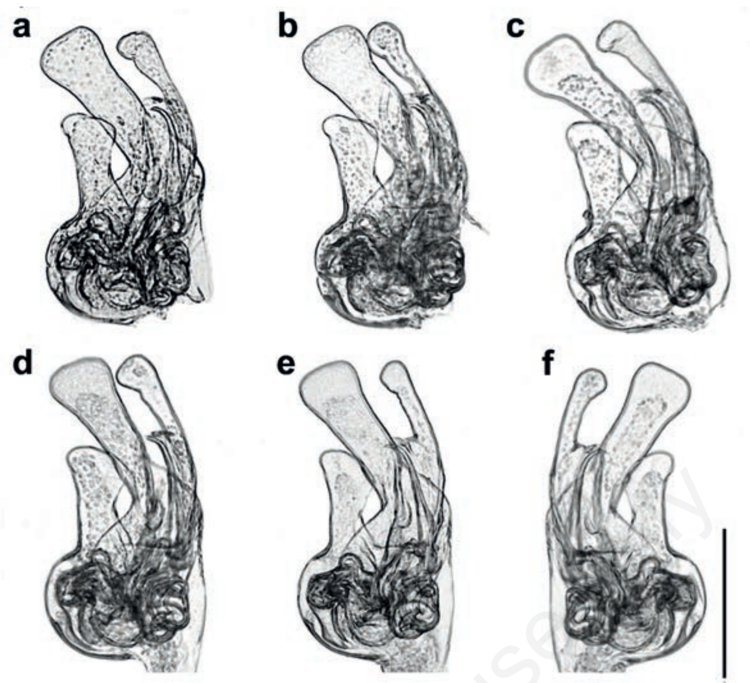

Fig. 12. *Ilyocypris dahlakensis* n.sp., light microscopy photographs of hemipenes. a,b) GR954-MZUF699; c, d: GR959-MZUF700; e,f) GR961. Scale bar: 100 µm.

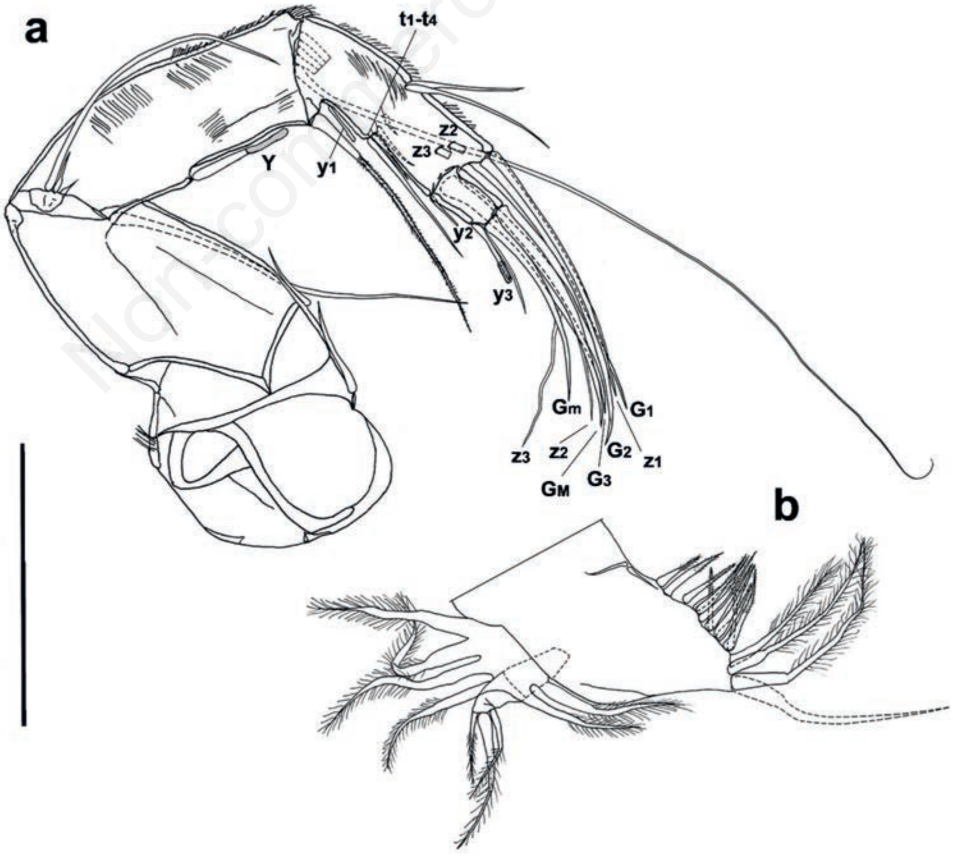

Fig. 13. A2 and T1 *Ilyocypris dahlakensis* n.sp., ♀, GR 960-MZUF703. a) A2; b) T1. Scale bar: 100 µm.

Differential diagnosis

The morphology of valves, with marked sexual dimorphism with a marked postero-ventral angle on ♀ and the additional closing mechanism on ♂ allows to easily differentiate the new species from its congeners. Also the soft parts, particularly the hemipenes, make *Ilyocypris dahlakensis* n.sp. clearly differentiable from the other species described in this genus. Kempf (2011) described the new genus *Juxilyocypris* of the ostracod family Ilyocyprididae for fossil specimens with very distinct sexual dimorphism in shell morphology. Although *I. dahlakensis* n.sp. displays such striking dimorphism, it lacks the prominently raised and bulged ridge along the anterior margin of both valves, a character typical of the genus (Kempf, 1967, 1975).

Etymology

The name of the species comes from the Dahlak archipelago, where the dry mud from which hatched the studied specimens was collected. The orthography of the specific name is different from that used for the spinicaudatan *Leptestheria dahalacensis* described by Rüppel (1837, sub *Estheria*) for the same islands, which he incorrectly referred to as "Inseln Dahalak" instead of Dahlak.

Distribution

Ilyocypris dahlakensis n.sp. is so far known from the pond D2 (type locality) and the neighbouring pond D3 on Dahlak Kebir Island, where dry mud was collected that once rewetted generated the specimens described here.

Remarks

Males and females were equally represented in the analysed samples.

COX1 SEQUENCES

Overall, six 675 bp-long COX1 sequences were produced by aligning both forward and reverse sequences for two *Cypris galefensis*, one *Chrissia martensi* n.sp., two *Ilyocypris dahlakensis* n.sp. and one *Plesiocypridopsis newtoni*. All novel ostracods COX1 sequences were deposited in the public database GenBank (Accession Numbers, A.N.s: PQ538284-PQ538289). In the frame of this work, we refrained from producing phylogenetic trees for all the investigated taxa since most of them lacked comparative COX1 sequences on GenBank.

DISCUSSION

This study reported the presence of five recent non-marine ostracod taxa, including two new species, from an area still poorly investigated with regard to this crustacean class.

Cypris galefensis and *Plesiocypridopsis newtoni* had already been reported from the Afrotropical region, as had the genus *Heterocypris* (with 15 species) as well as the genera of the two new species described here, *Chrissia* (with 17 species) and *Ilyocypris* (with four species) (Meisch *et al.*, 2024).

According to different authors (Savatenalinton, 2023; Zhai *et al.,* 2023), the taxonomy of *Chrissia* needs to be revised, as the morphological variability within this genus may indicate the presence of distinct lineages. The need for revision should probably be extended to the entire subfamily Herpetocypridinae (Kong *et al.*, 2014).

In the case of *Ilyocypris*, taxonomy is also rather problematic (Mazzini *et al.*, 2014; Smith *et al.*, 2019) by the generally similar carapace morphologies in the genus and the high intraspecific variability in valve morphology. Therefore, specific identification of living specimens is mainly based on the characters of the appendages, such as the length of the natatory setae on A2, the division of the penultimate segment of T2, and the number of setae on the penultimate segment of T3 (Meisch, 2000; Karanovic and Lee, 2013). For species with males, the hemipenis possesses the most useful characters for species identification, namely the shapes of the three lobes and the copulatory process, although the latter is rarely described (Smith *et al.*, 2019). In the case of *Ilyocypris dahlakensis* n.sp., some variability in the shape of the lobes of the hemipenis is evident (Figs. 11 d,e and 12), as is a marked sexual dimorphism in carapace shapes (Figs. 8 and 9) that is uncommon in the genus. The morphological plasticity of the valves and the possibility of sexually dimorphic species in this genus must therefore be given due consideration in palaeontological studies.

Also of interest are the differences in the morphology of the prehensile palps when comparing different populations of *Plesiocypridopis newtoni*, a species found mainly in the Palaearctic but also present in the Oriental region (Meisch *et al.*, 2024). The prehensile organs play an important role in the mating process and are considered diagnostic to distinguish between related species (Martens, 1991; Karanovic *et al.*, 2019). A similar case concerning the variability of these appendages among geographically distant populations was recently reported in *Cypridopsis vidua* (O.F. Müller, 1776), a species (or more likely a species-complex) with one of the widest geographical distributions among non-marine ostracods (Rossetti *et al.*, 2024).

As for the ecological characteristics of the two new ostracod species found in the Dahlak Islands during this study, very little information is currently available, apart from their obvious ability to produce resting stages and thrive in an arid area, and the tolerance to high solute concentrations. The analysed specimens of *Chrissia martensi* n.sp. were all females, none of which had sperms inside the carapace, thus indicating the possibility of parthenogenetic reproduction in this species; however, it cannot be excluded *a priori* that males of this species may exist. What reported above is quite common for the genus *Chrissia*, being relatively scarce the number of species for which males have been so far described. In the case of *Ilyocypris dahlakensis* n.sp., the balanced presence of both sexes suggests an exclusively (or predominantly) sexual reproduction.

The results of this study confirm that rehydration of diapausing stages in the laboratory is an effective method to describe the diversity of aquatic invertebrates in arid areas, where the presence of water is limited to short periods and hardly predictable.

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